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Baseline Level and Early Suppression of Serum HCV RNA for Predicting Sustained Complete Response to Alpha-Interferon Therapy

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The relationship between serum hepatitis C virus (HCV) RNA and the outcome of alpha-interferon (α-IFN) therapy in patients with chronic hepatitis C has important implications for therapeutic research and clinical care. Serum HCV RNA was tested for HCV genotype and quantified by a standardized reverse transcriptase–polymerase chain reaction assay as a measure of viral load in a cohort of 130 patients with chronic hepatitis C treated with α-IFN at a standard dose of 3 million units three times a week scheduled for 6 (n = 50) or 12 months (n = 76). Twenty-one of 126 evaluable patients (16.7%) developed a sustained complete response to α-IFN according to biochemical and virological criteria. The 3 pretreatment independent factors associated with a sustained complete response were a low baseline serum HCV RNA concentration, non-1 HCV genotype, and female sex. A multivariate logistic regression model, with pretreatment and month 1 variables, showed that a lower baseline serum HCV RNA concentration, female sex, and a greater suppression of RNA were the significant predictors of sustained complete response. The lowest baseline serum HCV RNA concentration was observed in patients with genotype 2 infection and the greatest decrease in HCV RNA from baseline to month 1 in those with genotype 3. The findings suggest that measuring HCV RNA in serum before and soon after begining treatment can be helpful for selecting patients who are most likely to have a sustained complete response to standard schedule of α-IFN and for identifying patients in whom alternative strategies should be examined. ***J. Med. Virol. 54:86–91, 1998.***

KEY WORDS: chronic hepatitis C; alpha-interferon; HCV RNA quantification; HCV genotype

INTRODUCTION

Hepatitis C virus (HCV) infection often leads to chronic liver disease, with chronic active hepatitis, cirrhosis, and hepatocellular carcinoma [Tong et al., 1995]. Alpha-interferon (α-IFN) is the only drug approved for treating HCV infections. Although there is as yet no "gold standard" for defining response, there is strong evidence that sustained virological response is an important endpoint. The continued absence of HCV RNA from serum after the end of therapy is associated with sustained normal serum alanine aminotransferase (ALT) activity and long-term histological improvement [Hoofnagle, 1994; Kasahara et al., 1995]. Conversely, persistent hepatitis C viremia predicts a later relapse after a sustained biochemical response [Chemello et al., 1996]. Unfortunately, sustained HCV RNA clearance occurs in only 10–15% of patients using current schedules, i.e., a dose of 3 million units three times a week for 24 or 48 weeks [Hoofnagle and Lau, 1996].

The limited effect of α-IFN on viral replication, the existence of potentially serious side effects, and the cost of treatment have led to suggestions that patients likely to derive a long-term benefit should be selected for treatment. Previous studies based on "in-house" techniques or first-generation molecular assays have shown, retrospectively, that a non-1 HCV genotype and a low pretreatment serum HCV RNA concentration are correlated with a sustained biochemical response

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[Yoshioka et al., 1992; Hagiwara et al., 1993; Lau et al., 1993; Magrin et al., 1994; Orito et al., 1994; Tsubota et al., 1994; Martinot-Peignoux et al., 1995; Yamada et al., 1995]. However, the clearance of HCV RNA from the serum of patients with sustained biochemical response was not assessed in these studies. It has also been reported recently that different HCV genotypes respond to α-IFN with different virological kinetics [Kohara et al., 1995]. Thus, early measurement of HCV RNA could also predict outcome. The optimal time at which to measure HCV RNA is unclear, and there are few data to guide clinicians as to which virological markers to use when designing clinical trials or for selecting later patients for treatment.

Therefore, we have attempted to determine the relationship between virological factors and the outcome of α-IFN therapy in subjects enrolled in a prospective study. Serum HCV RNA concentrations were measured by using a sensitive, commercially available reverse transcriptase–polymerase chain reaction (RT-PCR) assay, first on entry into the study and then during treatment. We assessed the influence of serum HCV RNA concentration, HCV genotype, and other clinical, biochemical, and histological parameters on sustained HCV RNA clearance after α-IFN treatment.

METHODS

Patients

The population studied consisted of 130 anti-HCV and HCV RNA-positive patients with chronic hepatitis C, eligible for interferon therapy, seen at the Liver Unit of Toulouse University Hospital, France, between November 1994 and July 1995. There were 71 men and 59 women, with a mean age of 45.1 years (range = 21–72). Blood transfusion and intravenous drug use were identified as potential source of infection in 43 and 33 patients, respectively. All patients had a serum ALT activity that had been elevated persistently for over 6 months and chronic active hepatitis without cirrhosis on liver biopsy (mean Knodell score = 7.1 ± 2.5). None of the patients was positive for hepatitis B surface antigen, antibody to the human immunodeficiency virus, or autoimmunity marker. None of the patients had ever received antiviral treatment.

All 130 patients were given subcutaneously 3 megaunits of α-IFN 2a (Roferon A; Roche Laboratories, Basel, Switzerland) three times a week for ≥3 months. Four patients were lost to follow-up and were not evaluated. Those patients with elevated ALT activity after 3 months of therapy were defined as nonresponders (n = 51), and the α-IFN treatment was stopped. Patients with normal ALT activity were given the same dosage for another 3 (n = 30) or 9 (n = 45) months, which was the schedule recommended in 1995 by the French Drug Agency for treating patients with chronic hepatitis C. Median follow-up after the end of treatment was 21 months (range = 9–29).

Virological Studies

Blood samples were collected without anticoagulant. Serum samples were prepared within 3 hours of blood collection, aliquoted, and stored at −80°C. Samples were thawed on ice only once before testing for HCV RNA. Serum HCV RNA concentrations were determined on two occasions during the pretreatment period by using the Amplicor HCV Monitor quantitative RT-PCR assay (Roche Molecular Systems, Branchburg, NJ); the geometric mean of these two measurements was defined as the baseline value and the standard deviation as the biological variability. Serum HCV RNA concentrations were measured at month 1 and month 3 after the initiation of α-IFN treatment. The HCV RNA copy number was assessed by using the manufacturer's instructions. The detection limit of the assay was 3 log copies/ml. The intraassay and interassay reproducibilities were similar at 0.14 log [Izopet et al., 1996]. Serum HCV RNA concentrations were also measured prior to the initiation of α-IFN therapy by the branched DNA (bDNA) probe assay (Quantiplex(R) HCV RNA, version 2.0, Chiron Corporation, Emeryville, CA). Serum was tested by using the Amplicor HCV qualitative RT-PCR assay (Roche Molecular Systems) [Young et al., 1993] after 1 month, 3 months, and at the end of therapy, and 1, 3, and 6 months posttreatment. The 5 major genotypes (1–5) and their subtypes were identified before treatment by using the Line Probe Assay (Innogenetics, Zwijndrecht, Belgium) [Stuyver et al., 1993] and were classified according to the nomenclature of Simmonds et al. [1994].

Statistical Analysis

Patients were assigned to one of two categories according to their response to treatment as determined by virological and biochemical criteria. The endpoint used was a sustained complete response defined as (a) the loss of HCV RNA from serum by PCR analyses at the end of treatment and on 3 occasions after the end of treatment (1, 3, and 6 months posttreatment) and (b) the normalization of serum ALT activity during treatment and its continuation throughout the follow-up period.

The serum HCV RNA concentrations were converted to log (base 10) values before analysis. Throughout the present study, HCV RNA concentrations are expressed as the log of the number of copies per milliliter. Specimens in which HCV RNA was undetectable were assigned values equal to half the reported detection limit of the RT-PCR assay (i.e., 2.7 log).

Proportions were compared by the chi-squared test or Fisher's exact test for expected values <5. Analyses of variance, two-sample t-tests, and logistic regression were used to test associations among baseline characteristics, including serum HCV RNA concentrations, HCV genotype, and clinical, biochemical, and histological factors. Tests for trend were used to compare sustained complete response rates according to the quartile of the baseline RNA measurements [Mantel and Haenszel, 1959]. Changes in RNA concentrations from baseline at months 1 and 3 were calculated as log (baseline RNA) − log (month 1 RNA) and as log (baseline RNA) − log (month 3 RNA), respectively. Logistic regression analyses were used to assess whether these

TABLE I. Serum HCV RNA Concentration and HCV Genotype According to the Characteristics of the Patients at Baseline

Characteristics	HCV RNA	*P*	HCV genotype 1	2	3	4/5	*P*
Sex							
Male	5.50 ± 0.74	0.32	36	8	17	6	0.13
Female	5.36 ± 0.85		29	16	10	3	
Age (years)							
⩽ 45	5.41 ± 0.70	0.66	26	9	25*	7	<0.001
> 45	5.47 ± 0.89		39	15	2	2	
History of blood transfusion							
Yes	5.46 ± 0.76	0.84	26	8	4	5	0.052
No	5.43 ± 0.81		39	16	23	4	
History of intravenous drug use							
Yes	5.57 ± 0.54	0.26	16	0	14**	3	<0.001
No	5.39 ± 0.87		49	24	13	6	
Alanine aminotransferase							
⩽ 2.6 normal value	5.39 ± 0.81	0.67	32	15	8	6	0.09
> 2.6 normal value	5.45 ± 0.80		31	9	18	3	
Aspartate aminotransferase							
⩽ 1.8 normal value	5.36 ± 0.80	0.5	31	16	10	6	0.20
> 1.8 normal value	5.46 ± 0.80		29	8	16	3	
γ-Glutamyl transpeptidase							
⩽ normal value	5.30 ± 0.88	0.024	32	17	21	3	0.019
> normal value	5.63 ± 0.62		32	7	6	6	
Ferritin (μg/l)							
⩽ 163	5.39 ± 0.81	0.48	25	13	19***	3	0.025
> 163	5.49 ± 0.80		39	9	8	6	
Knodell score							
⩽ 7	5.45 ± 0.76	0.7	33	13	14	6	0.67
> 7	5.40 ± 0.85		31	10	13	2	

*Genotype 3 vs. other genotypes, $P < 0.001$ (chi-square test).
**Genotype 3 vs. other genotypes, $P < 0.05$ (chi-square test).
***Genotype 3 vs. other genotypes, $P = 0.05$ (chi-square test).

virological measures independently predicted the likelihood of a sustained complete response.

RESULTS

Of the 126 evaluable patients enrolled in the study, 21 (16.7%) developed a sustained complete response to α-IFN using virological and biochemical criteria; 5/50 (10%) of patients treated for 6 months had a sustained complete response, as did 16/76 (21%) of those treated for 12 months ($P > 0.05$). Of the 105 remaining patients, 51 (40.5%) had elevated ALT activities after 3 months of therapy, 19 (15.1%) had a breakthrough, i.e., ALT increased during treatment after initial normalization, 32 (25.4%) had a biochemical relapse with an increase in ALT after treatment following an initial normalization, and 3 (2.4%) had a sustained biochemical response, but HCV RNA was not cleared from the serum after cessation of therapy. Ten patients stopped treatment because of adverse events at months 4 (n = 4), 5 (n = 3), 7 (n = 1), and 10 (n = 2).

Pretreatment Characteristics

Serum HCV RNA concentrations were assessed at baseline in two separate measurements, 1–3 months before α-IFN and immediately before treatment. Only three patients had values below the detection limit of the quantitative RT-PCR assay. The mean serum HCV RNA concentration was 5.44 ± 0.79 log copies per milliliter, and some values were as high as 6.61 log. The biological variability, expressed as the standard deviation of the copy number of the two baseline measurements, was 0.23 log. HCV genotype distribution was: 1 (65 cases), 2 (24 cases), 3 (27 cases), and 4/5 (9 cases); HCV RNA was untypable in one patient. The subtypes were 1a (14), 1b (40), 1 (11), 2a/c (23), 2b (1), 3a (24), 3 (3), and 4/5 (9). Patients with HCV genotype 1 infections had higher baseline serum HCV RNA concentrations (5.82 ± 0.40 log) than did those with genotype 2 (4.58 ± 1.04 log, $P < 0.001$), genotype 3 (5.46 ± 0.57 log, $P = 0.003$), or genotype 4/5 (5.10 ± 0.46 log, $P < 0.001$). The patients with HCV genotype 1 infection had similar baseline serum HCV RNA concentrations, whether infected with subtypes 1a, 1b, or 1 (data not shown). The relation between the baseline characteristics and the serum HCV RNA concentrations and HCV genotype is shown in Table I. Patients with normal Γ-GT activity had lower serum HCV RNA concentrations, but the association was no longer statistically significant after adjusting for HCV genotype. Patients with genotype 3 infection were younger ($P < 0.001$), had acquired infection more frequently by intravenous drug use ($P < 0.05$), and had lower serum ferritin concentrations ($P = 0.05$).

Predictive Variables at Baseline

When the patients were grouped according to the quartile in which their RNA values fell (Fig. 1), 37.5% of the patients in the quartile with the lowest viral load

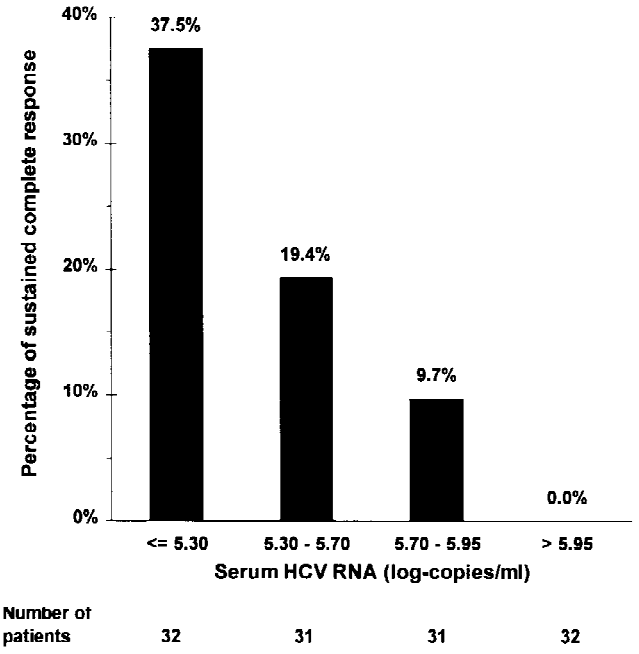

Fig. 1. Percentage of sustained complete response in the study cohort according to quartile of the serum HCV RNA concentration measured at baseline, with a *P* value calculated by the test for trend among RNA quartiles. The number of patients studied in each subgroup is shown below the graph.

had sustained complete response, as did 19.4% of those in the second quartile, 9.7% in the third, and none of those with the highest viral load ($P < 0.001$). The proportions of patients with sustained complete response according to HCV genotype were 1/65 (1.5%, genotype 1), 10/24 (41.7%, genotype 2), 8/27 (29.6%, genotype 3), and 1/9 (11.1%, genotype 4/5) ($P < 0.001$). Female sex ($P = 0.017$) and normal Γ-GT activity ($P = 0.02$) were the other pretreatment variables that were significantly associated with a sustained complete response. Logistic regression models were used to assess how well the baseline variables predicted the likelihood of sustained complete response, either individually (in a univariate analysis) or in combination (in a multivariate analysis) (Table II). Lower HCV RNA concentration, non-1 HCV genotype, and female sex were the three independent predictors.

Changes in Serum HCV RNA Concentration During Treatment

As shown in Figure 2, the patients who had a sustained complete response had lower initial RNA concentrations (4.72 ± 1.01 log) than did those who did not (5.58 ± 0.66 log, $P < 0.001$). The patients with a sustained complete response also showed a lower HCV RNA concentration after 1 month of treatment (2.78 ± 0.27 log) than did the other patients (4.56 ± 1.52 log, $P < 0.001$) and after 3 months of treatment. HCV RNA decrease in patients with a sustained complete response was −1.94 ± 0.96 log after 1 month and −2.02 ± 1.01 log after 3 months. Among the nonresponders, patients with elevated ALT activity after 3 months of treatment had a lower rate of HCV RNA decrease (−0.41 ± 0.59 at month 1; −0.62 ± 0.76 at month 3) than did those with breakthrough (−1.75 ± 1.08 at month 1; −2.01 ± 1.16 at month 3; $P < 0.001$) or those with relapse (−1.64 ± 1.08 at month 1; −2.02 ± 1.01 at month 3; $P < 0.001$).

TABLE II. Pretreatment Variables Associated With a Sustained Complete Response in Univariate and Multivariate Analyses

Variable	Odds ratio	95% Confidence interval	*P*
Univariate analysis			
Lower HCV RNA[a]	3.23	1.80–5.80	< 0.001
Non-1 HCV genotype	31.2	4.03–241.6	< 0.001
Female sex	3.47	1.25–9.64	0.017
Normal γ-GT activity	4.58	1.27–16.5	0.02
Multivariate analysis			
Lower HCV RNA[a]	1.95	1.01–3.77	0.045
Non-1 HCV genotype	20.0	2.38–168.3	0.006
Female sex	4.0	1.25–12.78	0.019

[a]The odds ratios are those associated with a 1.0 lower baseline concentration, in log copies per milliliter.

The kinetics of serum HCV RNA over the first 3 months of treatment were linked to the HCV genotype. Patients with genotype 3 infection showed a higher HCV RNA decrease at month 1 (−2.33 ± 0.93 log) than did those with genotype 1 (−0.78 ± 0.90, $P < 0.001$), genotype 2 (−1.20 ± 0.97, $P < 0.001$), or genotype 4/5 (−0.74 ± 1.00, $P < 0.001$). This difference persisted after adjusting for host variables associated significantly with genotype 3 infection (age, source of infection, and serum ferritin).

The responses to α-IFN during the first month, measured as the suppression of serum HCV RNA (i.e., a decrease in HCV RNA concentration or nondetectable HCV RNA by qualitative PCR) or the normalization of ALT activity, were included in a multivariate logistic regression model with the pretreatment variables associated with a sustained complete response (Table III). The three significant independent predictors of sustained complete response identified were a low baseline serum HCV RNA concentration, female sex, and a higher RNA decrease during the first month. After 3 months of treatment, among the 75 patients who had a normal ALT activity, 28 (37%) had a positive HCV RNA; none had a sustained complete response. All patients who had a sustained complete response had a normal ALT activity and a negative HCV RNA by quantitative and qualitative RT-PCR at month 3. The responses at 1 and 3 months predicted the outcome of both treatment groups (6 months and 12 months of α-IFN therapy).

DISCUSSION

The present study shows that the baseline concentration and kinetics of HCV RNA under treatment are related to the HCV genotype. Patients infected with genotype 1 had the highest baseline serum HCV RNA

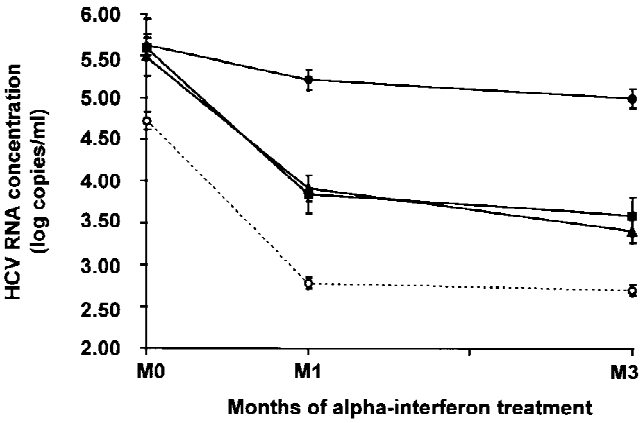

Fig. 2. Serum HCV RNA concentrations (mean ± SE) as determined by RT-PCR at baseline at month 1 of α-INF therapy and at month 3 of α-INF therapy in patients with no biochemical response (solid circle), with breakthrough (square), with relapse (triangle), and with sustained complete response (open circle).

TABLE III. Multivariate Logistic Regression Model for Predicting a Sustained Complete Response to Alpha-Interferon

Variable	Odds ratio	95% Confidence interval	*P*
Lower HCV RNA at baseline[a]	19.5	3.37–112.9	< 0.001
Female sex	6.69	1.83–22.88	0.004
RNA decrease from baseline[b]	11.36	2.17–57.41	0.004

[a]The odds ratios are those associated with a 1.0 lower baseline concentration, in log copies per milliliter
[b]The odds ratios are those associated with a decrease of 1.0, in log copies per milliliter.

concentrations and those infected with genotype 2 the lowest. Because the efficiency of PCR-based assay could be genotype dependent, leading to underestimation of genotypes other than genotype 1 [Ohno and Lau, 1996; Zeuzem et al., 1996], the pretreatment serum samples of our patients were also tested by using the bDNA version 2.0 assays, which is known to estimate HCV RNA accurately from all HCV genotypes [Lau et al., 1995; Detmer et al., 1996]. This assay indicated that patients with genotype 2 had significantly lower baseline HCV RNA concentrations than patients with any other genotype (data not shown). These results differ from those of two studies, one carried out on blood donors [Smith et al., 1996] and another on patients with chronic hepatitis C [Lau et al., 1996], where concentrations of HCV RNA were found to be similar across HCV genotypes 1–3. However, HCV RNA was assayed by using the bDNA version 1.0 in both studies, and correction factors were retrospectively applied for genotypes 2 and 3. The patients infected with genotype 3 had a higher HCV RNA decrease after 1 month of treatment (2.3 log reduction) than did patients infected with any other genotype (0.8–1.2 log reduction). This result suggests that the intrinsic sensitivity of the HCV genotype 3 to α-IFN is greater than that of the other genotypes or that the infected host is more sensitive to α-IFN. The patients with HCV genotype 3 infection were 10 years younger, had more frequently acquired infection by intravenous drug use, and had lower serum ferritin concentrations than did the other patients. But the fact that the association between the genotype and the rate of virus loss persisted after adjusting for age, source of infection, and serum ferritin points to the genotype 3 sensitivity.

Pretreatment variables associated independently with a sustained complete response were a lower baseline serum HCV RNA concentration, a non-1 HCV genotype, and female sex. The association between the baseline serum HCV RNA concentration and the virological response has also been found by multivariate analysis in other studies [Shindo et al., 1995; Pawlotsky et al., 1996]. In a multivariate regression logistic model with pretreatment and month 1 variables, the independent predictors were a lower HCV RNA at baseline, female sex, and a greater decrease in RNA from baseline. The absence of HCV genotype as an independent factor in this model is not surprising in view of the relationships between HCV genotype and the baseline level or short-term change in HCV RNA concentration. The favorable therapy outcome in patients with genotype 2 could be due to the low pretreatment RNA concentration of genotype 2. Similarly, the high rate of sustained complete response in patients with genotype 3 could be due to the low baseline serum HCV RNA concentration and the high intrinsic sensitivity of genotype 3 to α-IFN. In vitro models would be useful for investigating these viral characteristics. Logistic regression models including pretreatment variables and changes at month 3 were not constructed because there was a 0 value in one cell. None of the patients who had a positive HCV RNA after 3 months of α-IFN therapy had a sustained complete response. Thus, we believe that clearance of HCV RNA from the serum during the first months of therapy is a prerequisite for a sustained complete response. When this response is achieved, prolonged treatment probably increases the rate of sustained virological response. In our patients, the rate of sustained complete response was higher in the group treated for 12 months (21%) than in the group treated for 6 months (10%), although this difference was not statistically significant. A longer treatment with no detectable RNA in the serum may increase the likelihood of obtaining no detectable RNA in the liver at the end of treatment, and this is known to be a good predictor of a long-term response [Shindo et al., 1995]. Conversely, continuing α-IFN treatment at the same dosage for a total duration of 6 months or 12 months does not increase the rate of sustained virological response when there is no HCV RNA clearance from the serum during the first 3 months of therapy.

In summary, the present prospective study indicates that testing for HCV RNA in serum rather than measuring only ALT activity can be helpful for monitoring. The results suggest that patients with serum HCV RNA concentration greater than 6 log copies/ml, a HCV

genotype 1, and who do not rapidly lose HCV RNA from the serum after the initiation of α-IFN should be offered a treatment schedule other than the standard course of α-IFN. But suitable alternative strategies, including combination therapy, have yet to be evaluated by clinical trials.

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